

# Detailed protocol for mouse adipose tissue protein extraction

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An abbreviated version of this protocol was published in eLIFE in Mar 2017

Angiopoietin-2 in white adipose tissue improves metabolic homeostasis through enhanced angiogenesis

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## Detailed protocol

The Scherer lab, modified on 09/30/2019

### Reagents:

1. RIPA buffer (50 mmol/L Tris-HCl (pH 8.0), 0.25 mol/L NaCl, 5 mmol/L EDTA)
2. Triton X-100
2. Protease inhibitor cocktail from Roche, #11697498001
3. Optional: phosphatase inhibitors if phosphorylated proteins will be measured

### Equipment:

1. Qiagen TissueLyser II homogenizer
2. ThermoScientific microcentrifuge (temperature controlled)

### Protocol:

1. Harvest adipose tissues and snap freeze in liquid nitrogen.
2. Add 1 mL RIPA buffer (~100mg adipose tissue) with protease inhibitor and homogenize using the TissueLyser II (Qiagen) at the highest frequency for 3-5 minutes until clear. Keep on ice.
3. Spin down at 6,000 RPM for 15 min at 4°C.
4. Carefully remove the fat cake and resuspend the loose pellet. (Alternatively, use the pipette tip to penetrate the fat cake and transfer the solution and the pellet to a new tube).
5. Add Triton X-100 to a final concentration of 1%.
6. Incubate at 4°C for 30-60 minutes.
7. Centrifuge at 12,000 RPM for 15 min at 4°C.
8. Transfer the supernatant to a new tube.
9. Store the extracted samples at -80°C until further BCA concentration measurement and Western Blotting.

### Tips:

1. The initial volume of RIPA buffer can be added to 1.2 mL/100 mg tissue. Higher efficiency of depleting lipid will be achieved with larger volume of lysis buffer.
2. Step 8 and 9 can be repeated for twice to get rid of maximum amount of lipid.

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. An, Y. and Scherer, P. E.(2019). Detailed protocol for mouse adipose tissue protein extraction. Bio-protocol Preprint. [bio-protocol.org/prep13](https://doi.org/10.21203/rs.3.rs-3111111/v1).
2. An, Y. A., Sun, K., Joffin, N., Zhang, F., Deng, Y., Donzé, O., Kusminski, C. M. and Scherer, P. E.(2017). Angiopoietin-2 in white adipose tissue improves metabolic homeostasis through enhanced angiogenesis. eLIFE. DOI: [10.7554/eLife.24071](https://doi.org/10.7554/eLife.24071)

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